Fly Patterning

Pattern formation – establishment of spatial arrangements of cells, structures etc.
- Involves a 2 step process
  1. cells obtain a positional value or information
  2. cells interpret and respond to positional information according to genetic program, developmental history, and additional cues

Genetic analysis by Wieschaus & Nüsslein-Volhard
- Led to detailed understanding of developmental mechanisms controlling embryogenesis
- Revolutionized the field of developmental biology
- Validated the use of model systems (learned about human development too!)

Genetic analysis
- screened for embryo lethal mutants
- analyzed cuticles for pattern defects
- found mutants that affect AP axis determination, segmentation and segment identity
- found mutants that disrupt DV axis specification and identity

Patterning was more complex than models predicted and involved surprise mechanisms

Remember, the early fly embryo is a syncitium so establishing the axis must be accomplished without cell interactions (signaling) like with the Nieuwkoop center and organizers of vertebrate systems.

A-P axis
Specified by a developmental-genetic hierarchy
- Maternal effect genes → axis determination and morphogen gradients
- Gap genes → define broad domains along the AP axis
- Pair-rule genes → expressed in, and specify, every other segment (actually parasegment)
- Segment polarity genes → define AP polarity within each segment
- Selector genes → specify the identity of each segment—also known as homeotic genes, or segment identity genes

Themes:
- At each level in the hierarchy, gene expression is regulated by genes from higher levels, and by interactions among genes of the same level.
- Genes are regulated combinatorially—multiple transcription factors act positively and negatively and the net transcriptional activity at any time or place depends on the combination of factors acting.
Maternal effect genes
Two organizing centers, anterior and posterior.
The anterior determinant is **bicoid** and one of the posterior determinants is **caudal**
- **Bicoid** mRNA is localized at the anterior pole of the oocyte
- Bicoid is necessary and sufficient to specify anterior fate (mutants and RNA injections)
- At fertilization, bicoid is translated to a protein. The protein diffuses away from the site of synthesis, creating a gradient with the highest concentration at the anterior pole.
- Bicoid protein is a positive regulator of anterior fate. It is a transcription factor that promotes the expression of the anterior gap gene **Hunchback**
- Bicoid is a negative regulator of posterior fate. It is also an RNA binding protein that binds to **caudal** mRNA and prevents its translation.
- Caudal mRNA is evenly distributed throughout the syncitial embryo.
- At the anterior where [bicoid] is high, there will be no caudal produced, but in the posterior where there is little or no bicoid, there will be a high concentration of caudal protein produced.
- Caudal is another transcription factor that promotes the expression of posterior specific gap genes

**Bicoid** also acts as a **morphogen** to specify position along the AP axis
- Regulates the transcription of gap genes in a concentration-dependent manner
- Can act as an activator or a repressor depending on the concentration and promoter context
  - Different affinity for positive and negative promoter elements
  - At high concentrations, all the elements are bound
  - At low concentrations, only the high affinity sites are bound

**Gap genes**
- Identified by mutations that deleted groups of consecutive segments (ie. caused gaps).
- Encode transcription factors
- Embryonically expressed (often mistakenly referred to as zygotic expression).
- Expressed in broad domains
- **Hunchback** required for anterior segments
- **Bicoid** → Hunchback expression
- **Krüppel** required for thoracic segments
- Expression regulated by bicoid
  - High [BCD] → kr (low affinity repressor sites)
  - Low [BCD] → kr (high affinity activator sites)
  - Thus, expression is repressed in the head and promoted in the thorax
- Krüppel and Hunchback also regulate each other’s expression
  - Hb →| Kr ; in a hb mutant, Kr expression expands anteriorly
  - Kr →| Hb ; in a kr mutant, Hb expression expands posteriorly
Initial expression patterns are broad and fuzzy but become restricted and sharper. Thus, gap genes are regulated by maternal effect genes and by interactions among gap genes.

**Pair-Rule genes**
- Identified by mutations that deleted alternating segments (actually parasegments=posterior half of one segment and anterior half of next)
  - o Unit of genetic control $\neq$ anatomical units
- expressed in every other segment, the same segments that are deleted in the respective mutant
- expressed at the onset of cellularization, but while still a syncitium
- regulated by maternal effect genes, gap genes and by interactions among pair-rule genes

Initial expression is in fuzzy stripes—from maternal effect and gap gene regulation. Interactions among pair-rule genes sharpen stripes and make sharp borders between expression domains.

The combination of autoactivation and mutual exclusion (2 gene products inhibit the expression of each others’ genes so only one or the other will be expressed in any given cell) generate sharp stripes of expression.

autoactivation (the transcription factor encoded by a gene binds to its own promoter and activates transcription)
mutual exclusion (2 gene products inhibit the expression of each others’ genes so only one or the other will be expressed in any given cell)

\[ \text{e.g. ftz} \quad \text{and} \quad \text{eve} \]
\[ \text{eve} \rightarrow \text{ftz} \]

Any given nucleus will express either a lot of eve or a lot of ftz, but not both. Regions of fuzzy overlap between eve and ftz expression will resolve into a sharp boundary.

**Segment Polarity Genes**
- Expression begins in cellular stage
- Expressed in 14 bands (every parasegment)
- Expression pattern initiated by pair-rule genes but maintained by cell interactions.

Functions:
1. to reinforce parasegmental periodicity (pattern) and boundaries
2. Specify cell fates within each parasegment.

1. Involves cell-cell interactions
Parasegment boundaries defined by **wingless (Wg)** and **hedgehog (Hh)** expression.
(The textbook talks a lot about **engrailed**, which encodes a transcription factor that drives expression of Hh. We will just worry about Hh.)

The initial expression pattern is defined by pair-rule genes. Hh is expressed wherever Ftz or Eve is expressed. Wg is expressed wherever Ftz or Eve is not expressed (see fig 9.25).

Wg and Hh both encode secreted paracrine signaling proteins.
Wg-expressing cells also express Patched, the Hh receptor. Hh signal transduction activates transcription of Wg and represses transcription of Hh.

\[
\text{Hh} \rightarrow \text{Wg} \\
\mid \text{Hh} \\
\text{Wg} \rightarrow \text{Hh} \\
\mid \text{Wg}
\]

Hh-expressing cells also express Frizzled, the Wg receptor. Wg signal transduction activates transcription of Hh and represses transcription of Wg.

Reciprocal loop providing **mutual reinforcement** of gene expression patterns. Another mechanism to define sharp boundaries of gene expression and cell fate specification.

2. Wg and Hh also form diffusion gradients that specify cell fates (position) within a parasegment. (see fig 9.26).
Hh is expressed in and specifies the anterior cell of each parasegment
Wg is expressed and specifies the posterior cell of each parasegment

**Segment Identity**

Controlled by segment identity (aka homeotic, aka selector) genes.

Discovered through **homeotic mutations**. This is a mutation that causes the transformation of one structure to another homologous structure. (Homologs have evolutionarily related ancestry—both derived from a common ancestor structure).

Eg. Antennapedia causes the transformation of antennae to legs;
ultrabithorax causes the transformation of halteres to wings (T3 to T2).

Drosophila homeotic genes encode transcription factors. These belong to a family with related DNA binding domains called **homeodomains**. The DNA sequence that codes for the homeodomain is called a homeobox. Genes that encode homeodomain proteins are knicknamed **Hox** genes. It is important to note that not all homeodomain encoding genes give homeotic mutant phenotypes and that not all genes (especially in other organisms) that give homeotic mutant phenotypes encode homeodomain proteins. In other words, **homeotic and homeodomain are NOT synonymous**.

Segment identity gene expression is initially regulated by gap and pair-rule genes. Expression patterns are refined and maintained by interactions with other pair-rule genes. General rule is that posterior genes repress anterior genes.

Eg. The normal **Antennapedia** gene is expressed in T2 and specifies the identity of T2.
The Antennapedia mutant is a dominant gain-of-function mutant where the Antp gene is ectopically expressed in a head segment. This represses the normal Hox gene in that (more anterior) head segment and converts the identity to (more posterior) T2, causing the homeotic transformation of antennae to legs.

Eg. **Ultrabithorax** is normally expressed in T3, where it represses the expression of Antp. In the **ubx** loss-of-function mutant, the without Ubx repression, Antp expression
expands posteriorly into T3, transforming it to T2 identity. Thus the fly has 2 T2 segments and the halteres in T3 are transformed to wings like T2.

Segment identity genes control the expression of realizator genes—the genes that actually direct morphogenesis. An example is the distal-less gene that promotes leg formation.

**DORSAL-VENTRAL PATTERNING**

A class of mutants where discovered that were “dorsalized”, that is they showed dorsal cell fates all around the circumference of the embryo. One was named dorsal and is a loss of function mutant, therefore Dorsal is necessary for ventral fate. Dorsal maternal effect gene

Encodes a transcription factor Dorsal \rightarrow ventral genes (eg. snail) --| dorsal genes (eg. Decapentaplegic)

Dorsal forms a morphogen gradient
- Protein initially evenly distributed in cytoplasm
- Ventral signal from maternal follicle cells induces translocation into nuclei
- Get gradient with high [Dorsal] in ventral nuclei and no Dorsal protein in dorsally located nuclei.

Two signaling events are required to establish the D-V axis.
1. A signal from the oocyte to the dorsal follicle cells specifies dorsal follicle cell fate. The default fate for follicle cells is ventral. Without this signal, all follicle cells have ventral fates.
   - The oocyte nucleus migrates to an anterior-dorsal position.
   - Gurken mRNA associates with the nucleus
   - Gurken encodes a paracrine signaling protein (related to EGF) that signals to the overlying follicle cells
   - Torpedo is expressed in the follicle cells and encodes the receptor for Gurken (receptor tyrosine kinase related to EGF receptor).
   - Torpedo activates a signal transduction cascade that promotes dorsal follicle fate

2. Ventral follicle cells signal to the embryo, inducing translocation of Dorsal protein into the nuclei where it specifies ventral fate in the embryo.
   - The perivitelline space contains several proteases in inactive pro-peptide form.
   - Ventral follicle cells express factors that result in cleavage and activation of the first protease, which in turn cleaves and activates the second protease and so forth. Ie. the ventral cells trigger a protease cascade in the perivitelline space.
   - The protease cascade results in cleavage and activation of the Spätzle protein.
   - Activated Spätzle acts as a ligand for the Toll receptor in the outer membrane of the syncitial blastoderm (embryo).

The Dorsal protein is evenly distributed in the syncitial cytoplasm. It is bound by another protein, Cactus, which prevents Dorsal from entering the nucleus.
Toll activation activates a signal transduction cascade that results in phosphorylation of Cactus, which targets it for ubiquitin mediated degradation. Degradation of Cactus releases Dorsal to enter the nucleus where it activates genes that control ventral cell fate in the embryo. Closest to ventral midline, get highest level of signaling from ventral follicle cells and therefore highest level of Dorsal entering nuclei. Have a gradient of nuclear Dorsal concentration as move dorsally from ventral midline. Get concentration dependent regulation of D-V fate genes that specify different cell fates along the D-V axis (see figs 9.34 and 9.40).

**Cartesian coordinate model**
Each region has a unique combination of A-P and D-V fates, and gene expression patterns. This is conceptually similar to a Cartesian coordinate system like on a map. Eg. Salivary glands are promoted by the Hox gene *scr* but inhibited by the D-V fate genes, *dorsal* and *dpp*. Thus, salivary glands form in the *scr* segment in the region between *dorsal* and *dpp* expression.